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Mitochondrial-epigenetic crosstalk in environmental toxicology

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Abstract

Crosstalk between the nuclear epigenome and mitochondria, both in normal physiological function and in responses to environmental toxicant exposures, is a developing sub-field of interest in environmental and molecular toxicology. The majority (~99%) of mitochondrial proteins are encoded in the nuclear genome, so programmed communication among nuclear, cytoplasmic, and mitochondrial compartments is essential for maintaining cellular health. In this review, we will focus on correlative and mechanistic evidence for direct impacts of each system on the other, discuss demonstrated or potential crosstalk in the context of chemical insult, and highlight biological research questions for future study. We will first review the two main signaling systems: nuclear signaling to the mitochondria [**anterograde signaling**], best described in regulation of oxidative phosphorylation (OXPHOS) and mitochondrial biogenesis in response to environmental signals received by the nucleus, and mitochondrial signals to the nucleus [**retrograde signaling**]. Both signaling systems can communicate intracellular energy needs or a need to compensate for dysfunction to maintain homeostasis, but both can also relay inappropriate signals in the presence of dysfunction in either system and contribute to adverse health outcomes. We will first review these two signaling systems and highlight known or biologically feasible epigenetic contributions to both, then briefly discuss the emerging field of **epigenetic regulation of the mitochondrial genome**, and finally discuss **putative “crosstalk phenotypes”**, including biological phenomena, such as caloric restriction, maintenance of stemness, and circadian rhythm, and states of disease or loss of function, such as cancer and aging, in which both the nuclear epigenome and mitochondria are strongly implicated.

Keywords

Epigenetics; Mitochondria; Environment; Toxicology

I. Introduction

Crosstalk between the nuclear epigenome and mitochondria, both in normal physiological function and in responses to environmental toxicant exposures, is a developing sub-field of interest in environmental and molecular toxicology. Several prior reviews on this topic

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primarily summarized the known functions of the nuclear epigenome and mitochondria and briefly discussed the biological feasibility of system crosstalk. In this review, we will focus on correlative and mechanistic evidence for direct impacts of each system on the other, discuss demonstrated or potential crosstalk in the context of chemical insult, and highlight biological research questions for future study. We will not provide in-depth biological detail of the two systems, but instead will direct the reader to several other recent reviews for more detailed information. The basic components of mitochondrial function and epigenomic regulation are reviewed in [1].

In this review, we have taken an inclusive view of the nuclear epigenome in describing examples of crosstalk. Most definitions of the epigenome describe it as the collection of mitotically heritable regulatory marks that govern gene expression programs [2]. Newer definitions also include functional roles for the epigenome in regulating nuclear genome stability, as well as coding and non-coding RNA stability and function [2]. Classic examples of regulatory programs include persistent maintenance of cellular identity and transient but mitotically conferrable responses to environmental stimuli, such as induction of the insulin gene in response to high blood glucose [2]. The nuclear epigenome is regulated by covalent modifications to DNA (and RNA), a range of post-translational modifications to histone proteins (primarily through methylation and acetylation of lysine residues), methyl binding proteins that bind methylated DNA, and chromatin remodeling complexes that change DNA's three-dimensional structure and thereby permit or restrict access to transcriptional machinery [2]. In general, methylation of cytosines in DNA, or "DNA methylation", represses gene expression, although it may serve as an activating mark in some genetic contexts; histone lysine acetylation is generally permissive for gene expression and histone lysine methylation can be both repressive (for example, trimethylation of histone 3 lysine 9, or H3K9me3, and H3K27me3) and permissive (H3K4me3) [2]. Non-coding RNA, including long-noncoding RNA (lncRNA) and microRNA, are variably included in definitions of the epigenome; some definitions only include RNA that show mitotically persistent regulatory effects (for example, Xist lncRNA bound to the inactivated X chromosome in mammalian females) [3]. In this review, we include discussion of all non-coding RNA. We also include transient chromatin remodeling to activate or repress gene transcription in this review, although these events are unlikely to be mitotically stable.

The majority (1500–2000) of mitochondrial proteins are encoded in the nuclear genome, so programmed communication among nuclear, cytoplasmic, and mitochondrial compartments is essential for maintaining cellular health [4, 5]. Two main signaling systems exist: nuclear signaling to the mitochondria [**anterograde signaling**], best described in regulation of oxidative phosphorylation (OXPHOS) and mitochondrial biogenesis in response to environmental signals received by the nucleus, and mitochondrial signals to the nucleus [**retrograde signaling**] (reviewed in [6]). Both signaling systems can communicate intracellular energy needs or a need to compensate for dysfunction to maintain homeostasis, but both can also relay inappropriate signals in the presence of dysfunction in either system and contribute to adverse health outcomes [4, 5]. We will first review the two main signaling systems and highlight known or biologically feasible epigenetic contributions to both, then briefly discuss the emerging field of **epigenetic regulation of the mitochondrial genome**,

and finally discuss **putative “crosstalk phenotypes”**, including biological phenomena, such as caloric restriction, maintenance of stemness, and circadian rhythm, and states of disease or loss of function, such as cancer and aging, in which both the nuclear epigenome and mitochondria are strongly implicated. We present examples with evidence for specific mechanisms for epigenetic modification separately in sections on anterograde and retrograde signaling, but we note that similar mechanisms may operate in both signaling systems. Although environmental toxicants have been shown to impact mitochondrial homeostasis and the nuclear epigenome individually, there are very few empirical examples of specific toxicants that influence mitochondrial function through epigenetic mechanisms, or the nuclear epigenome through mitochondrial function – we will highlight these examples in relevant sections. We will avoid using the term “mitoepigenetics”, which is sometimes used to refer to mitochondrial-epigenetic crosstalk and sometimes to epigenetic modification of mitochondrial DNA. Figure 1 serves to illustrate the organization and major mitochondrial-epigenome interactions discussed in this review.

II. The nuclear epigenome in anterograde signaling

“Anterograde signaling” refers to biological signals sent from the nucleus to the mitochondrion. In most cases of anterograde signaling, the nucleus serves as a conduit for communicating cellular needs to the mitochondria, through upregulation of nuclear transcription factors that then trigger transcription of nuclear-encoded gene sets required for oxidative phosphorylation (OXPHOS) and mitochondrial biogenesis [6].

A number of nuclear factors are required for transcriptional activation of nuclear encoded mitochondrial genes, including transcription factors (TFs) nuclear respiratory factors 1 and 2 (NRF1, NRF2), peroxisome proliferator-activated receptors- α , - γ , and - δ (PPAR- α , PPAR- γ , PPAR- δ) [1, 7], and transcriptional co-activators peroxisome proliferator-activated receptor γ co-activator-1 α and -1 β (PGC-1 α , PGC-1 β), and PGC-1 related protein (PERC or PRC) [1, 8]. NRF target genes include subunits of electron transport chain (ETC) complexes required for OXPHOS, as well as components of mitochondrial replication and transcriptional machinery, including mitochondrial DNA polymerase- γ (*POLG*), mitochondrial RNA polymerase (*POLRMT*), and mitochondrial transcription factors (*TFAM*, *mtTFB I* and *mtTFB II*) required for mitochondrial biogenesis, mitochondrial DNA (mtDNA) replication, and mtDNA transcription [8]. Other nuclear receptors can interact with PGC-1 α to induce transcription of alternate gene sets or to provide tissue-specific ligands for induction of the same gene sets; for example, estrogen receptors, including ER- α , ER- β , and estrogen related receptor (ERR), bind PGC-1 α to induce mitochondrial fatty acid oxidation and OXPHOS [1]. Transcriptional control of nuclear-encoded mitochondrial genes is reviewed in [1, 8].

In this section, we will discuss the role of the nuclear epigenome in classic examples of anterograde signaling in normal mammalian cellular function, including nuclear regulation of OXPHOS/glycolysis and stimulation of mitochondrial DNA replication and biogenesis programs in response to damage and/or increased cellular energy needs. Evidence for control of transcription of nuclear-encoded mitochondrial genes by the nuclear epigenome includes direct control through chromatin remodeling initiated by pioneer transcription

factor binding or through DNA methylation of regulatory sequences, as well as indirect control through recruitment of chromatin modifiers by transcriptional co-activators. In addition, we will summarize provocative emerging evidence suggesting the presence and function of nuclear transcription factors in the mitochondria; although transcription factors are not mitotically heritable epigenetic marks, they are critical players, both causal and responsive, in epigenetic patterning and chromatin function. This section will end with a brief discussion of the ways in which nuclear dysfunction can trigger mitochondrial dysfunction. (See Figure 1, top panel.)

Nuclear regulation of oxidative phosphorylation and glycolysis

One well-established example of anterograde signaling that involves transient epigenetic changes is the nuclear response to glucose availability. The nuclear epigenome plays a critical role in this anterograde response. One clear example of this role is **direct remodeling of chromatin by pioneer transcription factors** to regulate gene sets required for oxidative phosphorylation (OXPHOS).

Genes required for OXPHOS are activated in response to low circulating insulin levels triggered by low glucose availability for glycolysis [1]. When insulin is low, un-phosphorylated Forkhead Box O (FOXO) TFs translocate to the nucleus and bind insulin response elements (IREs) in promoters of nuclear OXPHOS gene targets [1]. Activation of these gene targets requires local chromatin remodeling to enable recruitment of transcriptional machinery. Binding of “pioneer” transcription factors triggers opening of chromatin to enable binding and recruitment of additional factors necessary for transcription ([9], reviewed in [10]). For example, the TFs FoxO1 and FoxA1/2 function as pioneer factors at the insulin target genes *insulin-like growth factor-binding protein 1 (IGFBP1)* and *glucose-6-phosphatase (G6PC)* [10]. FOXO TFs’ pioneer capabilities are due to a highly conserved winged helix DNA binding motif; this variant of a helix-turn-helix motif in which the recognition helix is flanked by two polypeptide “wings” is structurally similar to the winged helix DNA binding globular domain of the linker histone protein [11, 12]. This structural similarity enables FoxO1 and FoxA1/A2 to stably bind recognition sequences within nucleosome particles and open linker histone-compacted nucleosome arrays *in vitro* [13, 14]. Cooperative binding of FoxO1 and FoxA1/A2 to adjacent IREs at both *IGFBP1* and *G6PC* in HepG2 cells triggers changes to histone PTMs and recruitment of RNA polymerase II (RNAPII) to stimulate target gene transcription [10]. Specifically, TF binding led to statistically significant increases in acetylation of lysine 27 on histone H3 (H3K27ac), a mark of transcriptionally active chromatin, at the *IGFBP1* promoter, as well as an apparent but not statistically significant increases in active marks acetylated H3K9 (H3K9ac) and trimethylated H3K4 (H3K4me3), as well as H3K4 dimethylation (H3K4me2), a mark of transcriptionally poised chromatin [11]. This cycle is reinforced by a concomitant increase in fatty acid oxidation, which takes place predominantly in the mitochondrion. A byproduct of glycolysis, which occurs primarily in the cytosol, is reduced NADH; decreased glycolysis leads to a buildup of oxidized nicotinamide adenine dinucleotide (NAD⁺), a co-factor for the sirtuin SIRT1 that deacetylates FOXO TFs and promotes their nuclear translocation, thereby enhancing upregulation of OXPHOS-related genes [1]. (SIRT1 is one of seven mammalian

NAD⁺-dependent sirtuins, each with discrete subcellular localization and functions. These are reviewed in [15].)

In contrast, when blood glucose is high, pancreatic insulin secretion is high and glucagon secretion is suppressed [1]. Insulin binds insulin receptors on target cells to activate phosphoinositide 3-kinase (PI3K), which activates protein kinase B (Akt/PKB), which, in turn phosphorylates FOXO TFs that are bound to IREs in promoters of target genes, attenuating TF binding to nucleosomes and decreasing transcription of key OXPHOS components and regulators [1, 16–19]. Increased glycolysis in the cytosol leads to increased levels of reduced NADH and reduced deacetylase activity of NAD⁺-dependent SIRT1, effectively sequestering FOXO TFs in the cytosol, promoting closed chromatin states at OXPHOS-related target genes [1,[19].

Stimulation of mitochondrial biogenesis and mitochondrial DNA replication programs

Mitochondrial biogenesis, defined as expansion of mitochondrial mass from existing organelles, occurs in response to cellular differentiation and environmental stimuli, and is driven by largely the same set of nuclear regulators that target OXPHOS-related genes [5]. Transcription factors and co-activators respond to physiological and nutritional signals that require increased mitochondrial mass, including cellular maturation during differentiation, thermogenesis and increased energy production needs, as well as to mitochondrial quality control, or “QC”, signals that monitor mitochondrial damage and dysfunction and stimulate the proliferation of healthy mitochondria and the degradation by mitophagy of dysfunctional mitochondria [5].

Two examples of epigenetic control of transcription of nuclear-encoded mitochondrial biogenesis include **indirect control through transcriptional co-activator recruitment of chromatin modifiers** to open chromatin and **direct control through DNA methylation** of regulatory sequences in target genes. Transcription factor NRF-1 may activate transcription of target genes indirectly, through recruitment by co-activator PGC-1 α of RNAPII and histone acetyltransferases SRC-1 and p33 that locally remodel chromatin to enable transcription initiation [20–22]. Nuclear transcription of mitochondrial DNA polymerase *POLG* is controlled directly by DNA methylation of a CpG island within exon 2; when this region is highly methylated, RNAPII binding and *POLG* expression are reduced [23, 24].

Epigenetic control of nuclear-encoded mitochondrial genes also has functional consequences downstream of altered gene transcription. *POLG* expression directly regulates mitochondrial copy number *in vitro* and active demethylation of the CpG island in exon 2 that controls *POLG* expression restores responsiveness to cellular differentiation signals in HSR-GBM1 glioblastoma cells, suggesting an additional target for DNA demethylating agents in cancer therapy [25]. In a follow-up study, Kelly et al confirmed that mouse oocytes, blastocysts and ESCs showed low (<10%) methylation, as compared to somatic tissues (>40%), but induced pluripotent stem (iPS) cells and somatic cell nuclear transfer (SCNT) ESCs showed >20% methylation, with correspondingly lower *Polg* expression and lower mtDNA copy number [24], suggesting abnormal or incomplete epigenetic reprogramming has functional mitochondrial consequences in artificially constructed pluripotent cells.

Nuclear regulators may influence mitochondrial gene expression

In addition to established evidence that nuclear TFs regulate nuclear encoded genes that impact mitochondrial metabolism and biogenesis, emerging evidence suggests that nuclear TFs may influence mitochondrial gene expression directly, via **import of nuclear TFs into the mitochondria** to regulate transcription of the mitochondrial genome [26]. Nuclear TF activity in mitochondria was first identified more than 15 years ago [26–28], but remains controversial, particularly due to concerns for laboratory contamination of purified mitochondrial cell fractions with nuclear TFs. Nuclear TFs with the strongest evidence for direct regulation of mitochondrial gene expression are the thyroid hormone T3 receptor p43 and CREB [26–28].

Both *in vitro* and *in vivo* evidence suggests that the 43-kDA thyroid hormone receptor isoform T3R- α 1, also known as p43, can directly bind regulatory sequences in the mitochondrial genome to activate transcription. The initial 1999 study by Casas *et al* reported that p43 was present in the mitochondrial matrix *in vitro*, had affinity for thyroid hormone T3 comparable to that of T3 nuclear receptor, and bound four mitochondrial sequences that had high sequence similarity to nuclear T3 response elements [29]. In addition, in the presence of T3, p43 increased levels of precursor and mature mRNA transcripts *in organello* and *in vitro*, an effect that was abolished by deletion of putative mitochondrial binding sequences [29]. Enriquez *et al* supported these results by demonstrating that thyroid hormone increased mtDNA transcription *in vitro* and *in vivo*; further, induced hypothyroid rats showed decreased protein occupation of mitochondrial regulatory sequences by DNA footprinting that increased to control levels with T3 treatment in the presence of p43 [30]. The p43 isoform was later identified by the Sterling laboratory as the adenine nucleotide translocase, but that result has not been repeated [31, 32]. Early evidence supports specific functional roles for p43 in the mitochondria. Protein levels of p43 increased transiently with cytochrome c during postnatal rat tongue development, supporting a role for p43 in regulating mitochondrial RNA synthesis in development [33]. Regulation of thyroid hormone signaling at the organelle level may allow for functional tuning in mitochondria; p43 overexpression in skeletal muscle led to an initial increase in mitochondrial mass in mice at 2 months of age, but triggered a dramatic drop at 23 months, likely due to oxidative damage secondary to p43's direct overstimulation of OXPHOS [34].

Nuclear transcription factor cAMP response element binding (CREB) protein binds cAMP response elements (CREs) in nuclear DNA; CREB may also bind mtDNA directly to regulate gene expression. CREB was present in the mitochondria isolated from cultured cortical [35, 36] and hippocampal neurons [36], adult rat brains [35] and liver [37], and labeled CREB was imported into biochemically-isolated mitochondria [37]. CREB is activated by phosphorylation of PKA and other kinases [26]. A self-contained CREB pathway exists in mitochondria (including cAMP, PKA, and CREB) that, when activated, induces direct binding of phosphorylated CREB to consensus CRE sequences *in organello* [32, 33, 34] and in the mitochondrial D-loop *in vivo* [38], as well as promoted mitochondrial gene expression and protein synthesis [37].

Nuclear transcription factors p53, ER- α , ER- β , STAT3, PPAR- γ , GR, and AP-1 have all been reported to localize in mitochondria [39–45], or bind to mitochondrial regulatory sequences [46–48], but evidence for functional roles for these TFs in mitochondria is limited [26, 39, 40, 49, 50].

Global regulators of the nuclear epigenome have also been identified in the mitochondria, although their specific mitochondrial functions are unclear. The maintenance DNA methyltransferase DNMT1 was isolated in mouse embryonic fibroblasts and HCT116 human colon carcinoma cells [51], and the *de novo* DNA methyltransferase DNMT3a was identified in mouse and human central nervous system mitochondrial fractions [52]. Mitochondrially-localized isoforms of ten-eleven translocation demethylase (TET) proteins have also been identified [53]. We discuss the evidence for functionally relevant epigenetic patterning in the mitochondrial genome in the section titled “*Transcriptional regulation of the mitochondrial genome*”.

In addition to mitochondrial import of protein products of nuclear-encoded mitochondrial genes, **nuclear-encoded regulatory RNAs** are imported into mammalian mitochondria. Well-established mitochondrial import of nuclear-encoded transfer RNA (tRNA) and ribosomal RNA (rRNA) suggest a mechanism for active transport of RNA into mitochondria [54]. The non-coding RNA components of RNase P, which cleaves polycistronic mitochondrial transcripts, and mitochondrial RNA processing enzyme (MRP), which helps initiate mitochondrial DNA replication, are encoded in the nuclear genome and imported to the mitochondria, as well [54]. Emerging evidence suggests that additional non-coding RNA are imported into mitochondria, although their functions are unclear. Small regulatory microRNA (miRNA) which are 21–22nt short RNA that act by antisense mechanisms to regulate multiple targets [55], with sequences that align to the nuclear genome are present in mitochondria isolated from mouse liver, human myotubes, and human HeLa and HEK293 cells [38]. Small regulatory piwi-interacting RNA (piRNA) and short interfering RNA (siRNA) that align to the nuclear genome have been identified in mitochondria from HeLa and HEK293 cells [54]. Ago2, a component of the nuclear RISC complex that processes miRNA, co-localized to the mitochondrial outer membrane, which suggests that it may serve as platform for site-specific regulation of bound target mRNA [54].

Nuclear epigenetic dysfunction can trigger mitochondrial dysfunction

Dysfunction in nuclear components that regulate nuclear-encoded mitochondrial gene expression or in nuclear genes with regulatory roles in mitochondrial metabolism can cause secondary mitochondrial dysfunction. For example, cells and animals with inactivated nuclear receptors, transcriptional co-activators PGC-1 α or PGC-1 β , and sirtuins show perturbed mitochondrial function [1]. In one particularly compelling example, loss of components of the SIN3-RPD3 histone deacetylase complex, a chromatin remodeling complex that comprises a transcriptional co-repressor (SIN3) and a catalytic histone deacetylase component (RPD3), compromises mitochondrial function [7]. Loss of either SIN3 or RPD3 in *Drosophila* cell culture leads to unviability characterized by up-regulation of mitochondrial genes encoded in both nuclear and mitochondrial genomes, increased mitochondrial citrate synthase activity, and decreased ATP levels, implying altered

mitochondrial activity, and increased mitochondrial mass, perhaps due to a compensatory biogenesis program in response to perturbed chromatin state and subsequent gene expression programs [7].

III. The nuclear epigenome in retrograde signaling

The corollary of anterograde signaling, “retrograde signaling” refers to biological signals sent from the mitochondrion to the nucleus. Retrograde signaling has been well documented in yeast and mammalian cell culture in response to a range of mitochondrial triggers [5, 56]. Evidence of transient or stable epigenetic changes as responses to causal mitochondrial signals in these pathways is limited but growing, and is an area of active research. In this section, first, we will review mitochondrially-derived metabolites and second messengers that serve as regulators or substrates for epigenetic marks, directly linking mitochondrial processes with nuclear epigenomic patterning. Next, we will discuss the role of the epigenome in the classic “retrograde response,” or compensatory nuclear response to declining mitochondrial function. We will conclude this subsection with a brief discussion on the ability of mitochondrial dysfunction to trigger nuclear dysfunction. (See Figure 1, center panel.)

Mitochondrially-derived metabolites and second messengers can drive epigenomic patterning

Mitochondrial metabolites can serve as substrates for reactions that modify DNA [4]. In addition, biomolecules whose abundance or redox status respond to mitochondrial activity can serve as metabolic sensors for nuclear chromatin responses [4]. These metabolites and “energy sensors” are essential for cross-compartment communication, so much so that some hypothesize an ancient evolutionary origin of these mechanisms [1]. Current empirical evidence is minimal and mechanisms for specificity of gene targets or gene sets that compose specific regulatory programs are largely unknown, but may be partly mediated by differential response to substrate levels by individual enzymes that modify specific residues or genomic regions and sequence specific co-activators that may recruit general epigenomic modifying enzymes to specific gene targets in response to environmental stimuli. In this subsection, we will review the metabolites and small molecules that may serve as substrates for epigenetic patterning, as well as highlight several examples supporting their role in retrograde signaling.

Acetyl co-enzyme A, or acetyl co-A, is primarily produced from pyruvate, the end product of glycolysis, but is also produced from fatty acid oxidation. Acetyl co-A can be further converted to citrate, which then enters the TCA cycle to produce ATP; if in excess, acetyl co-A can be used to synthesize fatty acids. The concentration of acetyl co-A is sometimes thought of as a cytosolic correlate for calorie availability [1]. Importantly for this review, acetyl co-A can also remain in the cytosol or be imported to the nucleus as a substrate for cytosolic or nuclear reactions. Acetyl co-A is a substrate for acetylation of proteins, including histones; when acetylated, DNA-bound histones have reduced affinity for DNA and trigger open chromatin states [1]. When glucose is limited, acetyl co-A levels decrease, protein acetylation increases, and chromatin is condensed [1]. One of the first clear

examples of acetyl co-A levels driving a transcriptional response was recently published, although the mechanism underlying target gene specificity is still unknown. In this example, lactate dehydrogenase was induced in activated T-cells to support aerobic glycolysis during inflammation; aerobic glycolysis led to a high concentration of cytosolic acetyl co-A, triggering increased histone acetylation and subsequent transcription of the nuclear encoded inflammatory gene *Interferon- γ* [57].

S-adenosylmethionine, or SAM, is produced from L-methionine and ATP [1]. Post-translational modification of nuclear histones with methyl groups and covalent modification of cytosines in nuclear DNA requires SAM as a substrate; however, SAM serves as a substrate for methylation of many non-histone proteins, as well, so shifts in SAM concentrations in the cytosol may or may not influence histone methylation levels [1]. Production of methionine from homocysteine is regulated by mitochondrial metabolism; the one-carbon metabolism component serine requires NAD⁺ for its biosynthesis, so SAM production is low when NAD⁺ is low [1]. Since NAD⁺ is high when glucose is low (See “*Anterograde signaling*”), SAM production is also high when glucose availability is low. In humans, folate and methionine metabolism, within the one-carbon metabolism cycle, regulates SAM production [58, 59]; folate metabolism also regulates SAM levels in yeast, and methionine metabolism in yeast, flies and mice [58, 59]. The specificity of DNA methylation and specific histone methylation changes in response to fluctuating SAM levels is reviewed in [60].

Histone demethylation involves two classes of reactions. FAD-dependent lysine demethylases like lysine-specific demethylase 1 (LSD1) use **flavin adenine dinucleotide**, or FAD, to oxidize methyl groups on lysine residues; the resulting FADH₂ is reoxidized by O₂ to generate hydrogen peroxide [1]. The Jumonji family of histone demethylases (Jmj-KDMs) use oxygen and **2-oxoglutarate** (2-OG), also known as α -ketoglutarate (α -KG), to catalyze removal of histone methyl groups, producing succinate in the process [1]. Succinate provides negative feedback by inhibiting Jmj-KDM activity [1]. 2-hydroxyglutarate, generated by further reduction of 2-OG in part by mutant isoforms of isocitrate dehydrogenase, also inhibits Jmj-KDM activity [61].

Nicotinamide adenine dinucleotide, or NAD⁺, is the oxidized form of the NAD⁺/NADH redox couple. NAD⁺ is a common reducing equivalent that reflects calorie availability because it is reduced to NADH in the cytosol as a result of cytosolic glycolysis but remains largely oxidized if glucose is low and energy is derived from fatty acid oxidation. Since fatty acid oxidation occurs primarily in the mitochondrion, it allows passive build-up of oxidized NAD⁺ in the cytosol (see “*Anterograde signaling*”) [1]. NAD⁺ is also a substrate for class III histone deacetylases, also known as sirtuins; since low glucose allows accumulation of NAD⁺, starvation activates sirtuins to deacetylate histones and DNA binding proteins to condense chromatin and reduce gene expression [1]. In addition, NAD⁺ was recently identified as a 5' mRNA modification, or rudimentary cap, in bacteria during transcription initiation, perhaps imparting additional structural complexity for regulation and affecting RNA stability [62]. Recently, this modification was identified in a eukaryote, *S. cerevisiae*, and data suggest that the moiety is added during initiation in both nuclear and mitochondrial transcription [62].

Reactive oxygen species, or ROS, like hydrogen peroxide, are byproducts of OXPHOS that are known to stimulate signaling pathways and can also influence epigenetic patterning [61]. In yeast, mitochondrial ROS can signal through Tel1p and Rad53p (homologs of mammalian DNA damage response kinases ATM and Chk2) to promote longevity [63]. This pathway subsequently inactivates Rph1p, a histone demethylase of sub-telomeric heterochromatin, leading to transcriptional silencing of telomeric genes [63]. (See review by Blajszczak and Bonini, “*Mitochondria targeting by environmental stressors: implications for redox cellular signaling*” in this Special Issue.)

In addition to ROS, **oxidative nuclear DNA damage** itself can serve as an epigenetic signal and perhaps a sensor of oxidative stress triggered by mitochondrial ROS production. Although traditionally considered to inhibit transcription by blocking TF binding or stalling nuclear RNA polymerase II, ROS-mediated oxidation of DNA to 8-oxo-7,8-dihydroguanine (8-oxo-dG) in gene promoters can serve as a signal for gene activation [64]. When 8-oxo-dG is formed in potential G-quadruplex forming sequences in a gene promoter, base excision repair by 8-oxoguanine DNA glycosylase OGG1 results in an abasic site that enables melting of the duplex and unmasking of G-quadruplex forming sequences [64]. These sequences can then adopt a G-quadruplex fold in which apurinic/apyrimidinic endonuclease 1 (APE1) binds but inefficiently cleaves the abasic site opposite the oxidized guanine and activates the adjacent gene [64]. This mechanism has been demonstrated at two genes, *VEGF* and *NTHL1*, *in vitro* [64], and is additionally supported by evidence of increased 8-oxo-dG levels and expression of genes involved in DNA repair, cell cycle, and stress response genes in response to infection or hypoxia [64]. These data suggest that repair of oxidized DNA base 8-oxo-dG in a potential G-quadruplex sequence can serve as a structural switch in response to an oxidative environment (or, in severe cases, oxidative stress) by triggering folding that enhances adjacent gene transcription [64].

Cytosolic **calcium** uptake into the mitochondria communicates cytosolic energy needs to the mitochondria, which stimulates nuclear gene expression responses to drive energy production [65]. Cells respond to environmental cues that trigger calcium mobilization to the cytosol from endoplasmic reticular stores [65]. Cytosolic calcium is imported to mitochondria, where it increases production of NADH via the TCA cycle in the matrix and subsequent consumption in the ETC, thus speeding energy production [65]. However, even cells with low basal energy requirements, like endothelial cells, which generally rely on glycolysis in the cytosol for energy, sequester calcium in mitochondria and commonly show repetitive calcium oscillations [65], suggesting calcium signals are important for processes other than ATP generation. Mitochondrial calcium can influence protein acetylation, including histone acetylation, by modulating activity and expression of sirtuins in mitochondria, nucleus, and cytosol [65]. Matrix NADH not consumed in the ETC is exported to the cytosol, where it represses NAD⁺-dependent sirtuin deacetylase function, yielding high histone acetylation and permissive chromatin structure for nuclear gene expression [65]. Histone H3 is a target of nuclear-cytosolic SIRT1 and SIRT2, and nuclear SIRT6; H3 acetylation fluctuates with *SIRT* expression [65]. Buildup of cytosolic NAD⁺, either through treatment with an NAD⁺ precursor or inhibition of the malate-aspartate shuttle, prevented H3 acetylation changes [65]. Persistent calcium oscillations trigger

increased nuclear expression of sirtuins *SIRT1*, *SIRT3*, *SIRT6*, and *SIRT4* [65]. Inhibiting mitochondrial calcium uptake abolished *SIRT1* expression changes [65].

It is worth noting here that nuclear gene expression and chromatin modifiers in the nucleus can influence levels of these metabolites, as well, indicating dynamic bi-directional crosstalk. For example, the SIN3 histone deacetylase complex binds promoters of genes involved in methionine catabolism, which influence SAM production, which in turn influences nuclear histone methylation levels [66].

Mitochondrial dysfunction can influence the nuclear epigenome

Mitochondria are maintained by several “quality control” (QC) mechanisms, including autophagy and selective mitophagy in response to damage. When QC mechanisms perform imperfectly, mitochondrial dysfunction accumulates over the cellular lifespan, requiring metabolic adaptation. The classic “retrograde response” involves signaling the nucleus to mount compensatory responses in the mitochondrial network [67]. In 1987, Parikh and colleagues first described varied nuclear-encoded transcripts in yeast strains depleted of intact mtDNA (rho⁰ strains) [68]; among these transcripts was one for CIT2, the peroxisomal citrate synthase gene, whose activation is now diagnostic for compensatory retrograde signaling [69]. Retrograde response genes in yeast, *Rtg1*, *Rtg2*, and *Rtg3*, induce different metabolic enzymes to enable alternative metabolic pathways in the presence of mitochondrial dysfunction (low ATP, high ROS, Ca²⁺ release) or mtDNA depletion [70, 71]. Despite the lack of identified Rtg orthologs in other species, subsequent studies reported induction of a wide range of metabolic and stress response target genes in rho⁰ strains, as well as in mammalian cells (5) treated with ETC inhibitors or uncouplers [72, 73], supporting retrograde signaling as a general adaptive response to loss of TCA cycle activity [6].

Defects in ETC proteins, mtDNA mutations, or mitochondrial copy number alterations resulting in membrane potential shifts can induce retrograde signaling to relay mitochondrial conditions to the nucleus [5]. Mitochondrial ROS may do this, too, by itself or through inducing mtDNA damage and a retrograde signaling response [5]. For example, mitochondrial DNA depletion *in vitro* results in aberrant DNA methylation of promoter CpG islands in the nucleus that were unmethylated in parental cell line; repletion of mitochondrial DNA partially rescues parental methylation patterns in these regions [74]. In addition, when a threshold of heteroplasmy, or mixture of mutant and wild type mtDNA, is reached, extensive reprogramming of nuclear DNA can occur mediated by retrograde signaling [4]. Heteroplasmy can result from deleterious mtDNA mutations that accumulate in response to environmental toxicants or a predominance of fission or fusion [4], as discussed in another review in this special issue [Meyer *et al.*, “Mitochondrial fusion, fission and mitochondrial toxicity.”] Mitochondria retrograde signaling arising from mtDNA mutations and alterations in mtDNA copy number has been implicated as a driver in tumorigenesis, although nuclear reprogramming in cancer makes it hard to definitively identify mitochondrial defects as drivers. Several studies using mtDNA cybrid models with unchanged nuclear backgrounds suggests that mitochondrial genome defects and retrograde signaling play a causal role in tumorigenesis [75]. Smiraglia *et al* reported that mitochondrial defects can lead to epigenetic

changes in the nucleus [76], perhaps contributing to the variable penetrance of many mitochondrial disorders. Target genes of the calcium/calcineurin retrograde signaling pathway show elevated levels of histone acetylation, which is rescued by silencing a retrograde signaling component, hnRNPA2, that is also a putative subunit of a chromatin remodeling complex. As noted above (*“Reactive oxygen species”*), ROS-mediated retrograde signaling extends lifespan in yeast via inactivation of histone demethylase Rph1 at subtelomeric heterochromatin, suppressing subtelomeric transcription [63].

The unfolded mitochondrial protein response, or UPRmt, involves retrograde signaling that triggers expression of mitochondrial proteostasis genes and alters expression of metabolic genes to adapt to stress [6]. In this response, unfolded or unassembled mitochondrial proteins are cleaved into peptide “distress signals”, which are transported into the cytoplasm. Through a mechanism that is not well understood, the TF ATFS-1, which, in absence of stress, is imported into mitochondria and degraded by LON protease, is blocked from mitochondrial uptake and instead translocates to the nucleus to trigger a transcriptional response [77]. Nuclear genes for chaperone proteins, glycolysis, and structural membrane proteins are upregulated, among others, and OXPHOS genes (ETC subunits, TCA cycle enzymes) are repressed, including those encoded in the mitochondrial genome [6]. The nuclear epigenome is ostensibly involved in changed expression levels of these specific genes (reviewed in [6]), but we are not aware of examples of additional responses in the nuclear epigenome.

Pathological retrograde signaling occurs when damaging nuclear compensatory responses are induced in the presence of mitochondrial dysfunction. Pathological retrograde signaling is important in MERRF (myoclonic epilepsy with ragged raw fibers) in which low ATP due to inefficient ETC triggers mitochondria to signal nuclei to induce proliferation of defective mitochondria, increasing mitochondrial mass in muscle in an attempt to correct the deficiency [5]. Another example of pathological retrograde signaling is maternally inherited deafness associated with the A1555G mtDNA mutation, in which rRNA methyltransferase mtTFB I-mediated 12S rRNA hypermethylation results in ROS generation that activates AMPK and pro-apoptotic nuclear TF E2F1, which was responsible for hearing loss in transgenic mice [5].

IV. Transcriptional regulation of the mitochondrial genome (or the “mitochondrial epigenome”

Although the majority of research questions focused on system crosstalk between the mitochondria and the epigenome examine transcriptional regulation of the nuclear genome, a subset of studies have focused on potential epigenetic control of transcription of mitochondrial genes. As mitochondria are bacterial in evolutionary origin, the structure and function of the mitochondrial genome are significantly different from those in the nuclear genome. Therefore, for context, we will first review replication and transcription of mitochondrial DNA, followed by a review of the evidence for chromatin-like condensation and covalent DNA modification of mitochondrial genomes. In addition, we will briefly discuss the epidemiological evidence for effects of environmental exposures on DNA

methylation patterns in human mitochondrial DNA. We will conclude this subsection with a brief review of small regulatory RNA, of both mitochondrial and nuclear origin, and their roles in regulation of mitochondrial transcription. (See Figure 1 bottom panel.)

Regulation of mitochondrial DNA replication and transcription

The human mitochondrial genome is circular, 1.6 kB in length, and codes for 13 proteins in the electron transport chain, as well as for two ribosomal RNAs (rRNAs) and 22 transfer RNAs (tRNAs) necessary for mitochondrial gene translation [74]. A single cell can contain hundreds or thousands of copies of the mitochondrial genome [26]. Mammalian mitochondrial RNA polymerase (*POLRMT*), DNA polymerase- γ (*POLG*), transcription factors *TFAM*, *mtTFB I* and *II*, and transcription termination factor *MTERF* are all nuclear encoded [5]. As mitochondria are derived from a symbiotic alpha-proteobacterium, transcription and translation are more similar to bacterial rather than eukaryotic systems [26]. There is only one non-coding region in the mitochondrial genome, termed the displacement loop, or D-loop, that contains 2–3 gene promoters, 2 origins of replication, and *cis*-acting regulatory elements [74]. Two established promoters are oriented in opposite directions, one on the light strand (light strand promoter, LSP) and one on the heavy strand (heavy strand promoter, HSP1), so named for their relative buoyant densities in cesium chloride gradients [78]. It is controversial whether the human mitochondrial heavy strand contains two distinct promoters (HSP1 and HSP2) or if two distinct transcripts are initiated from different locations within a single promoter [79]. Only one independent H strand promoter is known in murine cells [79]. Mitochondrial genes are transcribed in three polycistronic transcripts (MT-ND6, MT-CO1 and MT-CO2) that do not contain introns and are not spliced, but rather processed by RNase P and other proteins, which excise tRNA sequences that flank mRNA and rRNA sequences, known as “tRNA punctuation” [26, 78]. The HSP2 site produces a transcript that corresponds to nearly the entire heavy strand; the HSP1 site is ~100bp upstream of HSP2 and produces a transcript that contains the two rRNA genes only [78]. The LSP transcript encompasses nearly the entire genome, and truncated LSP transcripts serve as primers for mtDNA replication [78, 80]. There is preliminary evidence for D-loop transcripts, too; alignment of RNA-seq reads to H and L strands in mouse and human cell lines only accounts for 97–99% of transcripts [79], although there are no reports of transcripts aligning to the D-loop.

Mitochondrial promoters have variable activity under different conditions [79]. A wide range of mitochondrial transcript levels in different cell lines has been observed under different experimental conditions, and it is unclear whether transcript abundance differences represent biological or technical effects [79]. A current working model hypothesizes that the levels of nuclear-encoded transcription factor TFAM may serve as a trigger for promoter switching [79]. Mitochondrial maintenance program supplies OXPHOS subunits through high HSP2 activity at low TFAM, but when TFAM levels rise, a biogenesis program turns on, which requires more rRNA (HSP1 activity) and primers for mtDNA replication (LSP) [79]. ATP levels can also regulate promoter activity. ATP concentrations up to 100 μ M stimulate transcription from both HSP1 and HSP2, but further increases suppress only HSP1 but not HSP2 [79]. Suppression of HSP1 at high ATP could enable down-regulation of translation when no ETC activity increase is required [79]. Transcription termination factor

4.4% frequency expected by chance alone [53]. In nDNA, this lower CpG content is attributed to spontaneous deamination of methylated cytosines into thymines [53], which supports higher CpG frequency and lower CpG methylation in mtDNA as compared to nDNA. It is likely that mtDNA methylation levels will vary with mtDNA copy number, and mixed cell populations [53].

Maintenance DNA methyltransferase DNMT1 and *de novo* DNA methyltransferase DNMT3a, both of which methylate cytosines in nuclear DNA, have been identified in mammalian mitochondria, too. Mammalian mitochondria have mtDNMT1 activity; an isoform of nuclear DNMT1 containing a mitochondrial target sequence translocates to the mitochondria [51, 87]. Dnmt3a was first identified in mammalian mitochondria in a mouse motor neuron cell line [85] and from mouse brain and spinal cord [85].

In addition to methylation of CpG sites common in mammalian nuclear DNA, early evidence suggests mammalian mtDNA may also contain methylated cytosines at CpA and CpT sites [85]. Although not yet identified, mammalian mtDNA could feasibly contain methylated adenines, which induce gene expression when present in bacterial promoters [82].

Mitochondrial DNA methylation and environmental exposures

Although the presence of DNA methylation in mitochondria DNA is interesting, it does not imply functional relevance. Many invertebrates carry a small fraction of methylated DNA that does not appear to function in regulation of gene transcription. However, correlative evidence in mice and humans raises the possibility that mtDNA methylation responds to environmental exposures and that gene transcription could be regulated by methylation [79]. Levels of 5hmC, but not 5mC, decreased with age in mouse frontal cortex, concurrent with high mRNA levels for NADH dehydrogenase (Complex I) subunits and DNA methylation enzyme level changes [23]. Methylation of two CpG sites within the 12S ribosomal RNA gene was inversely correlated with subject age [23]. Decreased Dnmt3a levels and increased 5mC levels at the 16S rRNA gene were reported in spinal cord neurons and skeletal muscle myofibrils from transgenic mouse models of ALS; the authors suggested that this tissue-specific mtDNA methylation change could contribute to inflammation in ALS pathology [88]. Finally, perinatal exposure to commercial mixture of pentabromodiphenyl ether congeners known as BDE-47, a flame retardant mixture, was associated with mtDNA methylation of *Mt-co2* gene in mouse brain [53].

In humans, DNA methylation of two mitochondrial genes, *MTF-TF* and *MT-RNR1*, in peripheral blood cells was associated with exposure to metal rich particulate matter (PM₁₀) [53]. This agrees with the observation that blood chromium levels in chrome plating workers was negatively associated with methylation at the same two mitochondrial genes, *MT-TF* and *MT-RNR1* [53]. In contrast, another study reported that DNA methylation of *MT-RNR1* was positively associated with PM_{2.5} exposure [53]. Dietary consumption of olive and perilla oils was negatively associated with methylation of mitochondrial genes *MT-ND4L* and *MT-TR* but positively associated with methylation of *MT-RNR1* [53]. Increased mtDNA methylation has been observed in plasma platelets from CVD patients [53], and altered D-loop methylation was associated with insulin resistance in obese individuals [53], in retinal

microvessels of diabetic retinopathy patients [53] and in colorectal cancer tissues compared to non-cancerous tissues [53]. It is worth noting here that it is unclear how some but not all genes contained in a mitochondrial polycistronic transcript could be upregulated or downregulated. Perhaps the remaining genes are systematically degraded in the mitochondrial degradosome [79], but we are unaware of any regulatory mechanisms for this specificity in transcript-level response.

Mitochondrial non-coding RNA with mitochondrial targets

In addition to importing regulatory non-coding RNA from the nucleus (see “*Anterograde signaling*”), reasonably strong evidence supports mitochondrially-encoded miRNA [53] and lncRNA [53]. It has been known since the early 2000s that mtDNA codes for lncRNA [53]. The first characterized mt-lncRNAs include three antisense transcripts from the 16S rRNA gene [53]. Another mt-lncRNA contains the sequences of two validated miRNAs of mitochondrial origin, *miR-4485* and *miR-1973* [53]. In addition, RNA-seq of the mitochondrial transcriptome also revealed non-canonical primary transcript processing sites, novel antisense RNAs, and mitochondrial small RNA (21nt and 26nt) [79]. Steady state mRNA levels are inversely correlated with high antisense transcript levels, implying antisense transcript regulation of coding mRNA [79]. Initially available through the MitomiR online database [53], a detailed classification of non-coding RNA of different types transcribed by mtDNA in different cells and tissues is now available through the **Mammalian Mitochondrial Non-coding RNA Database** [53], which is enabled with BLAST search functions.

Since mitochondrial DNA structure and function have some similarities with bacterial systems, bacterial small RNA origins and modes of action may provide starting points for emerging research questions on human mitochondrial small RNA. Bacterial small RNAs (sRNAs) share features with miRNAs, their most obvious functional eukaryotic equivalents [55]. Most bacterial small RNAs act by antisense mechanisms on multiple target mRNAs and impact transcript translation or stability [55]. Bacterial sRNAs can be generated from 5' or 3' regions of mRNA encoding genes, referred to as “parallel transcriptional output”, or transcribed from coding sequence [55]. The RNA component of RNase P is a housekeeping sRNA in bacteria [55]; the catalytic ncRNA in mammalian mitochondrial RNase P is transcribed from nuclear DNA [26]. Some bacterial sRNA, like lncRNA in mammalian cells, act as protein sequestrators rather than antisense binders, by binding proteins and preventing them from functioning normally; others bind other regulatory RNA as decoys and indirectly affect target gene expression [55]. Riboswitches, or elements in the 5' UTR of mRNAs that can fold into two mutually exclusive RNA conformations triggered by ligand binding or responses to abiotic factors like pH or temperature, can activate or inhibit translation, induce transcriptional read-through or trigger premature termination [55]. Only a few *E. coli* and *Salmonella* sRNA are constitutively expressed; the vast majority is transcriptionally induced under specific conditions, usually under TF control [55].

V. Putative “crosstalk phenotypes”

Several prior reviews have noted that both mitochondrial and epigenomic systems are implicated in a range of well-established biological phenomena and disease states and suggested that these data are indicative of system crosstalk. In this section, we highlight four biological phenomena or disease states for which we found the most comprehensive evidence of potential crosstalk: (1) maintenance or induction of stemness in primary or artificially derived stem cells; (2) caloric restriction, aging and longevity; (3) circadian rhythms; and (4) cancer. Although we were unable to find mechanistic evidence of crosstalk for each functional example, we include them here as suggestive phenotypic connections between systems and to highlight potential areas for research expansion.

Maintenance or induction of stemness

Mitochondrial function is linked to fate and function of stem cells [89, 90]. In mice and humans, the post-fertilization zygote has initially high numbers of residual oocyte mitochondria that are immature, spherical, perinuclear, glycolytic, and have low mitochondrial membrane potential [91]. No mitochondrial biogenesis or mtDNA replication occurs in pre-implantation cells, so after each cell division, daughter cells contain fewer mitochondria than parent cells; therefore, early stage stem cells have relatively low mitochondrial mass relative to differentiated cells [91]. MtDNA replication begins in the late morula or blastocyst stages; at this point, mitochondrial morphology is elongated and networked and oxygen consumption and OXPHOS increase [92]. Early tissue-specific stem cells show similar patterns; primordial germ cells that later give rise to spermatozoa and oocytes have low mtDNA copy number [61]. Although they are glycolytic, pluripotent cells do need mitochondria; loss of mitochondrial function through excessive fission or POLG knockdown leads to loss of pluripotency [89]. Stem cells repurpose mitochondria by redirecting metabolic intermediates like pyruvate and 2-OG from ATP generation to anabolic pathways and post-translational protein modification in a process termed ‘cataplerosis’ [89]. Pyruvate is converted to acetyl co-A and then to citrate; citrate, unlike acetyl co-A, can be exported to the cytosol, where it can be converted back to acetyl co-A and used as a substrate for histone acetylation reactions [89]. Limiting citrate’s conversion to acetyl co-A by inhibiting ATP citrate lyase, triggers differentiation in myocytes; acetate supplementation impairs early differentiation and histone deacetylation [89]. ESC differentiation leads to decreased acetyl co-A levels and loss of specific histone acetylation marks (H3K9ac and H3K27ac), suggesting that acetyl co-A is critical for maintaining open chromatin regions in pluripotency [89]. In embryonic stem cells (ESCs), 2-OG drives histone demethylation (H3K9me3, H3K27me3, H4K20me3) and maintains pluripotency [89]. Pluripotency may rely on glycolysis, but other properties of stemness do not. Exposure of primordial germ cells to hypoxia induced glycolysis and reprogramming to EGC-like cells, but these EGC-like cells did not self-renew [93].

Reductions in mitochondrial DNA copy number, density, and distribution to recapitulate mitochondrial features of ESCs are required to induce pluripotency in generating iPSCs [89]. Nuclear reprogramming with stemness transcription factors (three Yamanaka factors Oct4, Sox2, and Klf4, plus Myc) in mouse embryonic fibroblasts reverted mitochondrial

networks into immature, spherical, perinuclear, cristae-poor structures with low membrane potential [94]. The addition of Myc, a regulator of energy metabolism and a proto-oncogene, to a Yamanaka three-factor cocktail to derive iPSCs increases glycolytic capacity of resulting cell colonies, as compared to the other three factors alone [95]. Functionally, this transition includes suppression of OXPHOS in favor of glycolysis; somatic source cells with greater glycolytic and lower oxidative activity reprogrammed more efficiently [95]. Reduced complex I and IV expression and increased complex II, III, and V expression are early reprogramming events that precede a shift to glycolysis and expression of pluripotency genes [89]; these data could indicate that metabolic reprogramming is an initiating event in induced pluripotency, implying that nuclear epigenomic reprogramming is a consequence of initial metabolic changes, and not vice versa. Temporal sampling demonstrated glycolytic gene potentiation prior to induction of pluripotent markers [94]. Increased glycolytic gene expression preceded pluripotent gene expression but was concurrent with global transcriptional and epigenomic reorganization, including demethylation of pluripotency gene promoters, during somatic cell dedifferentiation from an oxidative to glycolytic iPSC state [94]. Possibly, glycolysis could provide an energy spike to facilitate epigenomic resetting, priming the cell for pluripotent induction [94], which would implicate metabolic remodeling as a key factor in nuclear reprogramming.

Recent studies also implicate mitochondrial fission in stem cell maintenance and differentiation [96]. Treatment of MEFs with mdivi-1, or mitochondrial division inhibitor, a small molecule that selectively inhibits the self-assembly of dynamin protein DRP1 that mediates mitochondrial fission, reduced nuclear reprogramming efficiency by more than 95% [96]. Treatment of MEFs with mdivi-1 at early reprogramming stages, before iPSC colony formation, was sufficient to completely inhibit somatic cell reprogramming, suggesting low functional capacity of highly networked mitochondria, as compared to small, spherical stem cell-like mitochondria to reprogram nuclear DNA [96].

Early developmental dynamics, often termed “reprogramming,” in both mitochondria (see above) and the epigenome (genome-wide regulatory marks are erased and re-established in PGCs and during early embryonic development [97, 98]) suggests roles for both systems in the Developmental Origins of Health and Disease (DOHaD), or altered adult health and disease risk based on early life exposures that may disrupt reprogramming events. There is suggestive evidence of mitochondrial-epigenetic crosstalk in patients with imprinting disorders, including Beckwith-Wiedemann syndrome, characterized by overexpression of imprinted gene *insulin-like growth factor 2 (IGF2)* that could potentially suppress OXPHOS [1], Prader-Willi and Angelman syndromes, characterized by aberrant expression of E3 ubiquitin ligase gene *UBE3A*, small, dense brain mitochondria and reduced complex II activity [1], and Rett syndrome, characterized by aberrant expression or activity of the X-linked methyl binding protein *MeCP2* gene, mitochondrial structural aberrations and reduced complexes I, III, and IV in skeletal muscle [1].

Caloric restriction, aging and longevity

Caloric restriction, defined as 30–50% reduction of caloric intake in mice, leads to longer lifespans, lower body weights, lower blood glucose and insulin, and increased resistance to

oxidative, genotoxic and heat stress, as well as delayed aging-associated diseases, in a range of experimental systems [99]. These changes are reflected in inhibition of glycolysis, activation of gluconeogenesis in liver, inhibition of adipogenesis, and myoblast differentiation [26]. CR effects on life span are very similar across diverse eukaryotes, suggesting a general survival strategy [99]. Low calorie availability leads to a buildup of NAD⁺ in the cytosol, triggering SIRT deacetylase activity and subsequent nuclear chromatin condensation (See “*Anterograde signaling*” and review of sirtuin function in [15]). Caloric restriction of *Sirt1* knockdown mice does not extend lifespan [1], suggesting Sirt1 as a component of the causal pathway. Sirtuins directly affect mitochondrial function, too. Knockdown of nucleocytosolic *Sirt1* in mice leads to uncoupled, inefficient mitochondria [1] and knockdown of mitochondrial *Sirt3* in mice leads to hyperacetylated mitochondrial proteins and ~50% reduction in mitochondrial ATP production [1]. CR also increases mitochondrial biogenesis and energy efficiency, possibly through nitric oxide signaling [99]. Historically, CR was thought to antagonize ROS effects on chromatin, perhaps through hormesis [99]. Current evidence suggests two CR responses through chromatin: (1) mediating cellular adaptation through control of gene expression through TF modulation and epigenetic condensation of large parts of the genome; and (2) promoting genomic stability [99].

The circadian clock (See “*Functional links: Circadian rhythm*”) is closely tied to mitochondrial biogenesis (See “*Anterograde signaling*”) and caloric restriction [15]. Caloric restriction activates mitochondrial biogenesis and simultaneously resets circadian gene expression and feeding rhythms in mice [15]. Caloric restriction also increases the abundance and activity of SIRT3, which in turn increases fatty acid oxidation in the mitochondria [15]. Overexpression of *SIRT3*, as well as *SIRT1* and *SIRT6*, protects against high fat diet-induced metabolic defects, and ablation of these sirtuins accelerates defects [15]. Caloric restriction alters expression or activity of the same three sirtuins [9]. Feeding mice a high fat diet abolishes circadian oscillation of NAD⁺, suggesting secondary effects on circadian SIRT activity [15].

Circadian rhythm

Rhythmic changes in the epigenome are essential for control of circadian gene expression. Briefly, the core CLOCK complex, a heterodimer containing TFs BMAL1 and CLOCK, drives expression of a core set of circadian genes through histone acetylation activity, including negative transcriptional regulators of the CLOCK complex, Period (PER) and Cryptochrome (CRY) [1, 15]. SIRT1 deacetylates both histone (H3K9 and H3K14 at promoters of CLOCK controlled genes and circadian control components) and non-histone (to CLOCK complex proteins, and promote degradation of repressor PER2) proteins to regulate circadian rhythm [1, 15]. SIRT1 activity is circadian and is linked to NAD⁺ oscillations, which can be influenced by food intake patterns (See “*Anterograde signaling*”) [1] but are largely regulated by an internal feedback loop that is inherently circadian [15]. Nicotinamide phosphoribosyltransferase, or NAMPT, is the rate-limiting enzyme in the NAD⁺ salvage pathway and its decrease with age in mouse hippocampus is responsible for aging-related decreases in circadian activity of sirtuins [15]. SIRT1, BMAL1, and CLOCK bind the NAMPT promoter in a circadian manner [15]. These data suggest that

environmental disruption of metabolic timing, for example with irregular late-night meals, could directly shift cyclical NAD⁺ availability and gene expression oscillations.

Nuclear-cytosolic SIRT1 is not the only sirtuin that regulates circadian rhythm. Nuclear SIRT6 and mitochondrial SIRT3 play important roles and rely on NAD⁺ availability for their function, too. Circadian genes regulated by SIRT6 are largely independent of those regulated by SIRT1 and peak expression of each gene set occurs in different circadian phases [15]. SIRT3 regulates circadian mitochondrial function and oxidative capacity [15]. Hepatic and serum metabolites show circadian oscillations in humans and mice, and lysine acetylation of mitochondrial proteins show circadian patterns [15]. Knockdown of *Bmal1* in MEFs and mice decreases oxygen consumption, fatty acid and glucose oxidation and decreased liver mitochondrial NAD⁺, with increased protein acetylation and inhibition of SIRT3 targets; this effect is rescued with supplementation of mutant mice with an NAD⁺ precursor [15].

Cancer

In the 1920s, Warburg and Cori described high glucose consumption and high lactate excretion from cancer cells due to aerobic glycolysis, or glycolysis in the presence of oxygen [90]. This is an uncommon phenomenon: normal, healthy cells commonly use OXPHOS in the presence of oxygen and glycolysis in the absence of oxygen [90, 100]. The “Warburg effect” is not consistent across cancer types, though; mitochondria across tumor types and in different tumor subpopulations have different bioenergetic strategies [100]. In one example, slow growing rat hepatoma cells were oxidative but highly proliferative hepatomas were glycolytic [100]. Metabolic flexibility is common in cancer: only ~17% of total ATP in various cancers and cancer cell lines is derived from glycolysis, but in some tumor types can be as low as 0.31% or as high at 64% [100]. Likely reasons for metabolic reprogramming as a key to uncontrolled proliferation include sustaining high proliferation in hypoxic conditions and evading apoptosis as a result of reduced mitochondrial function [100]. Mitochondria may be drivers of carcinogenic metabolism; as compared to intact 143B.TK-osteosarcoma cells, cybrids with osteosarcoma cell nuclear DNA but mtDNA from non-cancer cells showed increased ATP synthesis, oxygen consumption and respiratory chain activities and reversal of oncogenic characteristics [23].

Metabolic flexibility extends beyond the cell into the local environment. In the reverse Warburg effect, the tumor microenvironment is glycolytic and feeds cancer cells [100]. Cancer-associated fibroblasts produce lactate in response to cancer cell-secreted hydrogen peroxide, which triggers fibroblast mitophagy, autophagy, and cellular catabolism with a loss of mitochondrial function and switch to anaerobic glycolysis [100]. The resulting lactate is exported to the extracellular space and is used to fuel oxidative metabolism in neighboring cancer cells [100]. This cancer cell-fibroblast relationship mimics one known between neurons and astrocytes; glucose-hungry astrocytes are glycolytic and astrocytes secrete lactate to fuel OXPHOS in neurons [100]. It is likely that solid tumor cores are hypoxic and glycolytic, and actively proliferating cells on the periphery likely use the excreted lactate for OXPHOS, leading to a symbiotic relationship between these two cell sub-populations [100]. Emerging evidence suggests that tumors have hypoxic regions with high glucose uptake and

aerobic glycolysis, and other well-vascularized regions use other carbon sources for OXPHOS [61].

Although glycolysis is common in healthy pluripotent stem cells, not all pluripotent cells are metabolically similar [90, 101]. Hypoxic conditions in some stem cell niches may drive cells, especially quiescent cells, to use anaerobic glycolysis; high proliferative rates may push the cell to aerobic glycolysis [101]. Culture at low oxygen improves survival and proliferation of stem cells *in vitro*; together with the knowledge of highly variable *in vivo* oxygen concentrations, oxygen may have regulatory properties as a signal for cell proliferation [101]. Some pluripotent stem cells show an uptick in glycolysis on differentiation to multipotent cells [101]. ESC differentiation to proliferating neural stem cells results in a decline in OXPHOS and a slight increase in glycolysis, which is inhibited only on terminal differentiation to neurons [101]. Although growing evidence supports aerobic glycolysis in enhanced biosynthesis to satisfy proliferative needs, this property may have other functions in stem cells; inhibiting glycolysis attenuates reprogramming of dividing somatic cells to iPSCs without affecting cell proliferation [101]. In addition, some of the glycolytic enzymes that are thought to promote tumor growth have non-metabolic effects that contribute to proliferation, for example pyruvate kinase isoform PKM2 cooperation with β -catenin to activate cell cycle genes, like *MYC* and *Cyclin D1*, in addition to glycolytic genes; this additional function is necessary for the Warburg effect and tumor growth [101]. One recent study suggests that the Warburg effect might regulate gene expression in colon cancer cells by causing butyrate accumulation and subsequently inhibiting histone deacetylases [101].

The nuclear epigenome responds to mitochondrial and metabolic changes in cancer. Cancer cells depleted of mtDNA show altered methylation patterns at 2–9% of CpG islands surveyed, comparable to 2–5% alterations during stem cell differentiation [1]. In three of four cases, removal of mtDNA from cancer cells led to nuclear DNA hypomethylation; when mtDNA was reintroduced, 30% of hypomethylated sites became remethylated [1]. In addition, heterogeneity in aberrant epigenomic and metabolic properties in primary cancer cells can provide a platform for selection to act on subclones; cells with greater epigenetic accessibility and therefore higher expression of genes in the pentose phosphate pathway, which generates NADPH, may be more successful at metastasizing [102]. Distant pancreatic ductal adenocarcinoma metastases showed large scale reprogramming of chromatin modifications in both gene-rich euchromatic domains (ECDs) and large organized heterochromatic H3K9-modified regions (LOCKS) and a co-evolved dependence on the oxidative pentose phosphate pathway [102]. Analysis of primary and metastatic sub-clones from an individual pancreatic adenocarcinoma patient revealed that the metastatic sub-clone was less differentiated, more resistant to oxidative stress in a glucose-dependent manner, and had higher NADPH concentrations than its match [102]. These data suggest a metabolic-epigenetic pathway link that enhances tumorigenic fitness during evolution of distant metastasis [102].

Normal tissues with some properties of cancer cells under hypoxia or other conditions may be useful in identifying metabolic and epigenomic drivers of individual characteristics of cancer cells. A normal physiological correlate of metabolic and epigenetic shifts in

carcinogenesis is trophoblast differentiation in the developing placenta, a highly proliferative tissue [103]. In normal placental development, progenitor villous cytotrophoblasts differentiate into either extravillous cytotrophoblasts (which further differentiate to an invasive phenotype) or multinucleated syncytiotrophoblasts, accompanied by a move away from a proliferative, immature phenotype [103]. Differentiation of CTBs to an invasive phenotype has been likened to epithelial-mesenchymal transition (EMT), or de-differentiation and acquisition of cancer-like properties like invasiveness, in cancer [103]. Differentiation to either mature cell type involves DNA methylation shifts, including hypomethylation of an intragenic region in the *death domain-associated protein 6 (DAXX)* gene, which can be counteracted with low oxygen conditions [103]; loss of DMR methylation is correlated with increased *DAXX* expression during differentiation [103]. *DAXX* is a histone chaperone that mediates deposition of H3.3 at heterochromatic regions of the genome and represses invasion in lung cancer by blocking EMT [103]. *In vitro* culture of CTB or ST in low oxygen increased methylation at this DMR, which correlates with delayed differentiation [103]. This same DMR is consistently differentially methylated in placenta from pre-eclamptic pregnancies [103], in which oxygen availability to the placenta is restricted.

Future directions in “crosstalk phenotypes”

In this section, we’ve provided short summaries of intriguing biological phenomena that have clear connections with both mitochondrial function and dynamic profiles in the nuclear epigenome. However, detailed mechanistic evidence for functional and physiological outcomes of mitochondrial-epigenetic crosstalk is critical for establishing this communication channel as a driver in any of these phenotypes. In many of these examples, one of the two systems is considered by the authors to be “primary,” and evidence in the secondary system is presented as consequential or as bystander effects, although which system is designated the driver system is inconsistently applied across laboratories. As this sub-field of mitochondrial-epigenetic crosstalk grows, it is our hope that future collaborative work will employ a “systems biology” framework with a focus on the interdependence of these two systems to addressing existing and emerging research questions.

VI. Conclusion

The emerging field of mitochondrial-epigenetic crosstalk includes well-established phenomena, like anterograde signaling pathways, as well as new retrograde pathways, and expanded understanding of the roles both systems play in phenotypes like caloric restriction and cancer and regulation of circadian rhythm and stem cell maintenance. The separate but related field of transcriptional regulation of mitochondrial DNA is even newer, but is already drawing interest in biomarker development from environmental epidemiologists. As understanding of communication between these systems under normal physiological conditions grows, we will be able to ask more targeted and meaningful mechanistic questions about how environmental exposures in different windows of development can dysregulate this communication or function of either compartment and the implications for long term cellular and organismal health.

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Figure 1.

Crosstalk between mitochondria and the nuclear epigenome. **1. The nuclear epigenome in anterograde signaling [top panel.]** Transcriptional activators can trigger chromatin remodeling to enable transcription of nuclear-encoded mitochondrial genes that control oxidative phosphorylation (TCA cycle genes, electron transport cycle genes) directly, in the case of pioneer transcription factors, or indirectly, via co-activator recruitment of chromatin modifying proteins. DNA methylation of regulatory sequences in nuclear-encoded mitochondrial genes, most notably DNA polymerase- γ (*POLG*), can regulate transcription of these genes and downstream mitochondrial phenotypes, such as mitochondrial DNA copy number, in the case of *POLG*. Emerging evidence suggests that nuclear regulators, including transcription factors, DNA methyltransferases, ten-eleven translocation (TET) demethylases, and non-coding RNA may be exported from the nucleus and imported to the mitochondria, where they may directly impact transcription of the mitochondrial genome. **2. The nuclear epigenome in retrograde signaling [center panel.]** Mitochondrially-derived metabolites or second messengers, including S-adenosylmethionine (SAM), acetyl co-enzyme A (acetyl co-A), nicotinamide adenine dinucleotide (NAD⁺), flavin adenine dinucleotide (FAD), reactive oxygen species (ROS), calcium ions (Ca²⁺) and 2-oxoglutarate (not shown for space) can influence DNA methylation and histone tail post-translational modifications, including acetylation and methylation, in the nuclear epigenome. Mitochondrial DNA depletion or heteroplasmy impact nuclear reprogramming in embryogenesis, and retrograde response pathways that are activated in response to mitochondrial dysfunction trigger nuclear chromatin responses to enable mitochondrial adaptation. **3. The mitochondrial epigenome [bottom panel.]** Emerging evidence implicates DNA methylation of the mitochondrial genome in direct regulation of mitochondrial transcription. In addition, compaction of the mitochondrial genome into nucleoids may function to repress transcription, analogously to nuclear heterochromatin, and non-coding RNA encoded in the mitochondrial genome (distinct from imported nuclear ncRNA) may regulate mitochondrial transcription.